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Metabolic differences between cold stored and machine perfused porcine kidneys: A ^{1}H NMR based study

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ABSTRACT

Hypothermic machine perfusion (HMP) and static cold storage (SCS) are the two methods used to preserve deceased donor kidneys prior to transplant. This study seeks to characterise the metabolic profile of HMP and SCS porcine kidneys in a cardiac death donor model.

Twenty kidneys were cold flushed and stored for two hours following retrieval. Paired kidneys then underwent 24 h of HMP or SCS or served as time zero controls. Metabolite quantification in both storage fluid and kidney tissue was performed using one dimensional ^{1}H NMR spectroscopy. For each metabolite, the net gain for each storage modality was determined by comparing the total amount in each closed system (i.e. total amount in storage fluid and kidney combined) compared with controls.

26 metabolites were included for analysis. Total system metabolite quantities following HMP or SCS were greater for 14 compared with controls (all $p < 0.05$). In addition to metabolic differences with control kidneys, the net metabolic gain during HMP was greater than SCS for 8 metabolites (all $p < 0.05$). These included metabolites related to central metabolism (lactate, glutamate, aspartate, fumarate and acetate).

The metabolic environments of both perfusion fluid and the kidney tissue are strikingly different between SCS and HMP systems in this animal model. The total amount of central metabolites such as lactate and glutamate observed in the HMP kidney system suggests a greater degree of *de novo* metabolic activity than in the SCS system. Maintenance of central metabolic pathways may contribute to the clinical benefits of HMP.

1. Introduction

Hypothermic Machine Perfusion (HMP) and Static Cold Storage (SCS) are the two methods of kidney preservation that are used widely in clinical practice during the time period between organ retrieval and implantation [16]. A key concept for both preservation modalities is that cellular metabolism, and therefore cellular metabolic requirements, are minimised in these hypothermic conditions and the rate of metabolism reported to be about 5–8% at temperatures below 4 °C [29] with a similar decrease in oxygen requirement [1].

The superiority of HMP over SCS is well documented [4,17,22,23,27] but the mechanisms by which this occurs are not clear. Improvement in flow dynamics, with fall in the intra-renal resistance is likely to be one factor but the additional metabolic support derived from the circulation of nutrient-containing perfusion fluid may also help preserve organ function and have a beneficial effect [7,30].

Metabolomic analyses of preservation fluid during HMP using 1D-^{1}H NMR (One-dimensional proton nuclear magnetic resonance) spectroscopy, by groups including our own, have demonstrated this to be reproducible and highly specific for metabolite identification and quantification [2,10,24]. However, surprisingly, to our knowledge there are no studies that have sought to compare the metabolomic profiles, or metabolome, of HMP and SCS kidneys.

Porcine kidneys are widely used in transplantation studies owing to their similar physiological and anatomical properties to human organs [9,11]. In addition, the metabolic profiles during

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periods of HMP for porcine and human kidneys are comparable [24], with a correlation between metabolite profiles during storage and post transplant outcome [2]. For HMP preserved human kidneys, the metabolic profile from perfusates of immediate graft function kidneys differs from those with delayed function [10] and reinforces the concept that significant metabolism occurs during HMP and that metabolism reflects functional outcome.

The aims of this study were twofold. Firstly, to determine the distribution of metabolites between the two different compartments (fluid and tissue) during the organ preservation period. Secondly, to determine the total amount of each metabolite within HMP and SCS kidneys systems after 24 h of organ storage and through comparison with control kidneys, the metabolic changes that occur.

2. Methods

2.1. Animal research

Abattoir/slaughterhouse pig kidneys (F.A. Gill, Wolverhampton, UK) were used and no animals were sacrificed solely for the purposes of this study, negating any need for ethical board approval. Experiments were performed on 22–26 week old male 'bacon weight' pigs, weighing 80–85 kg. All experiments were performed following the principles of laboratory animal care according to NIH standards. Animals were sacrificed by electrical stunning and exsanguination. Initial organ preservation was performed following organ retrieval and occurred within 14 min of death, replicating deceased cardiac death (DCD) donor conditions. Kidneys were cold flushed (4 °C) with 1 L SPS-1 (UW) solution at a pressure of 100 mm Hg. Organs were then stored at 4 °C in SPS-1 for 2 h to replicate the clinical period of organ transportation.

2.2. Experimental groups

Paired kidneys were randomly allocated to receive either HMP or SCS for 24 h. HMP kidneys were perfused with 1 L of KPS-1 using the LifePort Kidney Transporter 1.0 (Organ Recovery Systems, Chicago, IL). (Perfusion pressure 30 mm Hg). SCS Kidneys were submerged in 1 L of fresh chilled SPS-1 solution with a surrounding ice bath. Preservation fluid was sampled for each kidney at baseline and 2, 4, 8, 12, 18, and 24 h. After 24 h, organs were rapidly dissected and tissue samples (1 cm^3 sections) flash frozen and stored (−80 °C). All experiments were performed in a cold room (4 °C) to ensure consistency.

2.3. Control kidneys

To ascertain metabolism during SCS or HMP storage conditions, baseline values prior to storage conditions were needed (time 0). Large volume tissue sampling precludes effective organ perfusion and therefore 'Control kidneys' were used to establish baseline metabolite levels. These were (n = 6) flushed and cold transported in identical fashion to experimental kidneys and tissue samples obtained as described above (i.e. not subjected to 24 hr of SCS or HMP).

2.4. Sample processing and metabolite quantification

NMR samples were prepared from storage fluid by mixing 150 μL of 400 mM (pH 7.0) phosphate buffer containing 2 mM DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid) and 8 mM imidazole with 390 μL of each fluid sample and 60 μL of deuterium oxide (D_2O) to reach a final phosphate buffer concentration of 100 mM and a final DSS concentration of 500 μM. After mixing, the 600 μL samples were pipetted into 5 mm NMR tubes, sonicated and centrifuged. Technical replicates of samples (×3) were prepared for each timepoint.

For cell extract studies, 500 mg of renal cortex was manually cryo-homogenised in liquid nitrogen. 5.1 ml of both methanol (−80 °C) and chloroform was added to the powdered tissue and samples diluted with 4.65 ml of dH_20 at 4 °C. Samples were centrifuged to separate into polar and non-polar layers and 1.5 ml of the upper polar layer was dispensed into a cryovial and dried. Three technical replicates were performed for each tissue sample. Dried polar residue was then dissolved in 390 μL of dH_20 and 210 μL of buffer solution as described above.

The protocol used for ^1H NMR analysis has been described previously [10,24]. Briefly, this entailed processing on a Bruker AVII 500 MHz spectrometer, acquisition of one dimensional spectra and then metabolite identification and quantification using Matlab based 'Metabolab' software [18] and Chenomx 8.1 (ChenomxInc) software respectively. Metabolites were deemed to be present if they exhibited non-ambiguous spectral patterns or their presence deemed biologically plausible and confirmed on ultra performance liquid chromatography mass spectrometry. Any metabolites that were present in different concentrations in the SCS and HMP fluid (e.g. glucose, gluconate, mannitol, adenine, adenosine etc.) were excluded from comparative analysis. Metabolite quantifications were corrected to allow for sample dilution with sample buffer. When determining concentrations of metabolites using Chenomx, the researchers were blind to the storage group. Quantification of the total amount of metabolite in the storage fluid, tissue and total system was calculated based upon the weight of the kidney at time of sample acquisition and final volume of storage fluid.

2.5. Statistical analysis

For each timepoint, three results were obtained (technical replicates) and the median value used. For comparison of SCS and HMP conditions, analysis was performed using Wilcoxon paired signed rank test (two tailed) as one kidney from each pair was subjected to each condition and normality was not consistent on prior analysis. When comparing SCS or HMP with control kidneys, Mann-Whitney u test (two tailed) was used, as these were non-paired samples. Data were reported as median concentrations and interquartile (IQ) range. All analysis was performed using GraphPad Prism version 6.00 for Mac OS X, GraphPad Software, La Jolla California USA, with $p < 0.05$ deemed to be indicative of statistical significance.

3. Results

Metabolic optimisation of cadaveric kidneys is a potential target to improve the function of kidneys for transplantation. This study seeks to establish the degree of metabolism, if any, that occurs in the two widely used methods of kidney organ storage prior to transplantation (HMP and SCS).

The total quantity of each metabolite after 24 h of either HMP or SCS was calculated using ^1H NMR methods and compared with control organs to determine the net metabolic change during each storage method.

We found evidence of metabolite production for both storage modalities with 14 metabolites present in significantly greater quantities in the HMP or SCS system compared with controls (all $p < 0.05$) (Table 1) (Fig. 1, Fig 1(Suppl)). There were significantly more metabolites with a net increase in the HMP system (13/14) compared with the SCS system (7/14) ($p = 0.033$).

Eight of the metabolites were significantly elevated in the HMP system compared with both the control and SCS systems (all $p < 0.05$), indicating a greater degree of metabolite production.